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Agmatine: Biological Role and Therapeutic Potentials in Morphine Analgesia and Dependence

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ABSTRACT

Agmatine is an amine that is formed by decarboxylation of L-arginine by the enzyme arginine decarboxylase (ADC) and hydrolyzed by the enzyme agmatinase to putrescine. Agmatine binds to several target receptors in the brain and has been proposed as a novel neuromodulator. In animal studies, agmatine potentiated morphine analgesia and reduced dependence/withdrawal. While the exact mechanism is not clear, the interactions with N-methyl-D-aspartate (NMDA) receptors, α2-adrenergic receptors, and intracellular cyclic adenosine monophosphate (cAMP) signaling have been proposed as possible targets. Like other monoamine transmitter molecules, agmatine is rapidly metabolized in the periphery and has poor penetration into the brain, which limits the use of agmatine itself as a therapeutic agent. However, the development of agmatinase inhibitors will offer a useful method to increase endogenous agmatine in the brain as a possible therapeutic approach to potentiate morphine analgesia and reduce dependence/withdrawal. This review provides a succinct discussion of the biological role/therapeutic potential of agmatine during morphine exposure/pain modulation, with an extensive amount of literature cited for further details.

KEYWORDS: agmatine, morphine, opioids, analgesia, withdrawal

INTRODUCTION

Agmatine is an amine that is formed by decarboxylation of L-arginine by the enzyme arginine decarboxylase (ADC) and hydrolyzed by the enzyme agmatinase (agmatine uryl hydrolase) to putrescine. After an initial report in 1994,[1] several laboratories confirmed the presence of agmatine in the brain and its interaction with several target receptors such as α2-adrenergic, imidazoline, and NMDA receptors.[1-4] These observations suggested that agmatine may have functions of a novel neurotransmitter/neuromodulator.[5] Using specific antibodies to agmatine,[6] we have shown by immunocytochemical studies that in the brain agmatine (1) is stored in the perikarya of a specific population of central neurons,[7] and (2) is found in small vesicles of axon terminals[8,9] that form synaptic contacts and is presumably costored and released with traditional transmitters/modulators, including l-glutamate and arginine vasopressin.[9] Agmatine is synthesized by a mammalian form of ADC[10,11] whose complementary DNA (cDNA) sequence has been recently identified.[12] Agmatine can be degraded by agmatinase in the brain[13] and by diamine oxidase in peripheral tissues.[14] The physiological role of agmatine in normal brain function is still unknown, in part because of the absence of adequate pharmacological tools to manipulate its synthesis and degradation. Moreover, since agmatine has several molecular targets and acts as an antagonist in most targets, it has been difficult to evaluate the function of endogenous agmatine in the whole organism. However, as discussed below, studies of the actions of exogenous agmatine have identified several intriguing neurally relevant functions of the amine that are of potential therapeutic importance. Agmatine administered intrathecally, locally or systemically, reduces the neuronal injury produced by excitotoxins,[15,16] global/focal ischemia,[17-19] spinal cord injury,[17,20] and hypoxic ischemic injury.[21] Another notable effect of agmatine is its ability to reduce chemically and electrically induced convulsive seizures.[22-24] Agmatine has also been proposed as an adjunct in the treatment of several chronic pain syndromes, and as effective in facilitating the action of morphine while profoundly reducing the development of tolerance.[25] Agmatine administered intrathecally or intraperitoneally (IP) blocks the development of morphine tolerance[20,25] and inhibits naloxone-precipitated signs of morphine withdrawal in rats.[26-29] Agmatine also has notable effects on learning behavior in fear-conditioning models[30,31] and antidepressant-like effects in depression models.[32-34]

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EXOGENOUS AGMATINE, MORPHINE, AND ANALGESIA

Several studies have reported the in vivo effects of agmatine on morphine analgesia/dependence, nociceptive responses, neuronal injury, and other behavioral effects in several animal models, as recently reviewed by Nguyen et al.[29] The first study, from the Pasternak laboratory, showed that agmatine at the dose of 10 mg/kg (subcutaneous) potentiated morphine analgesia and prevented the development of tolerance to chronic morphine.[25] Subsequently, several studies have confirmed the effects of agmatine on morphine pain tolerance and withdrawal symptoms with doses ranging from 1 to 25 mg/kg.[26,35,36] Most intriguing is agmatine's ability to potentiate the analgesic effect of morphine while also reducing the withdrawal symptoms after chronic exposure.[35,37] Thus, acute injection of agmatine by the peripheral route increases the analgesic effects of morphine and reduces tolerance to repeated morphine injection. Agmatine has been shown to reduce symptoms of withdrawal from morphine as measured by physical dependence in animal models. The reduction of central symptoms required the expression of functional neuronal nitric oxide synthase activity.[38] In related studies, intravenous agmatine was shown to evoke the escalation of fentanyl, but not cocaine, self-administration.[39] Besides potentiating morphine analgesia, agmatine, by itself, also has noted effects on nociceptive responses.[20,27,40-43] For example, agmatine can act like an antihyperalgesic agent in reducing the mechanical and inflammation-induced hypersensitivity to pain stimulation.[20] It is also important to point out that in all animal studies reported, agmatine had no effect on normal behavior, motor activity, or cardiovascular parameters and had no other toxic effects in normal animals in doses up to 100 mg/kg.

Molecular Targets of Agmatine Action

As agmatine does not bind to opiate receptors, the analgesic effects and the reduction of withdrawal to morphine are not likely to be mediated by a direct effect on opiate receptors.[44] However, agmatine interacts with several other target proteins that could mediate its effects. Agmatine binds to NMDA receptors and acts as an antagonist at NMDA receptor channels,[8,16,45-47] and NMDA antagonists are known to block opioid withdrawal symptoms.[48] Agmatine also binds to α2-adrenergic receptors, and agonists of α2-adrenergic receptors have been known to inhibit opioid withdrawal. The activation of α2-adrenergic receptors by agonists like clonidine inhibits dependence and withdrawal. While agmatine was discovered because of its ability to bind to α2-adrenergic receptors,[1] several subsequent functional studies reported that agmatine is not an agonist at this site.[4,49] Moreover, administration of α2-adrenergic agonists like clonidine, while blocking opiate withdrawal, causes sympathetic inhibition and reduction in arterial pressure.[50-52] In several animal models, agmatine, administered intracerebro-ventricular (i.c.v.) or IP, has not been shown to lower arterial pressure,[53-55] thus ruling out the possibility of α2-adrenergic receptor activation in this action of agmatine. Thus, the actions of agmatine on morphine pain response and withdrawal are most likely mediated by its ability to block NMDA receptor channels.[56] Meanwhile, other studies have suggested that the blockade of NMDA receptors may not be the only mechanism by which agmatine regulates glutamatergic neurotransmission during acute or chronic morphine exposure. Agmatine has been shown to modulate presynaptic calcium channels,[57,58] and such an effect could lead to lower glutamate release after acute agmatine injection. Our recent results indicate that acute agmatine administration reduces extracellular glutamate during pentylenetetrazole (PTZ)-induced convulsive seizures as measured by in vivo microdialysis.[59] Thus, agmatine may also regulate the release of glutamate during morphine withdrawal.

In initial behavior studies in rats and mice, symptoms of withdrawal from morphine, induced by naloxone, were reduced by agmatine when it was injected with naloxone,[25,35] probably because the agmatine directly inhibited NMDA receptors. More recently, we showed that chronic injection of agmatine during morphine exposure (lasting 7 days), but not at the time of inducing withdrawal by naloxone, substantially reduced the withdrawal symptoms.[37] Since agmatine was not present during the induction of withdrawal, direct inhibition of NMDA receptors could not be involved in this mode of agmatine action. Therefore, it appears that agmatine, administered during the development of morphine dependence, blocks the events leading to a hyperexcitable state of the neurons during chronic morphine exposure, thereby reducing withdrawal. This idea was conceived based on several reports indicating intracellular effects of agmatine. These effects include inhibition of cellular proliferation in kidney and vascular smooth muscle cells,[60,61] inflammatory signaling in macrophages and glial cells,[62-64] and morphine-induced cAMP superactivation in NG108-15 cells[65] and the rat brain.[37]

The cellular and molecular mechanisms for opiate tolerance/dependence and withdrawal have been fairly well documented. Electrophysiological and neurochemical studies have indicated that a hyperexcitable state of neurons in the locus coeruleus (LC), the ventral tegmental area (VTA), and the nucleus accumbens after chronic morphine exposure contributes to dependence and withdrawal. These changes occur because of a cycle of molecular events of higher phosphorylation and gene expression, resulting in adaptive upregulation of the cAMP system after chronic morphine abuse.[66] This upregulation has been shown in in vitro model systems including NG108-15 cells and cells transfected with μ-opioid receptors, as well as in in vivo animal models.[67-70] The resulting higher cAMP causes increased protein kinase A (PKA) activity, which phosphorylates several target proteins, including tyrosine hydroxylase (TH) and cAMP response element binding protein (CREB). The phosphorylated CREB subsequently acts as a transcription factor increasing the expression of several proteins, including adenylate cyclase and TH.[71] There is also evidence that initial suppression of cAMP production by morphine could increase the expression of specific subunits of PKA.[71] All these molecular changes initiate the cycle of events resulting in higher TH, adenylate cyclase, PKA, and several phosphorylated proteins, including membrane sodium channels, causing the hyperexcitable state of the neurons. We hypothesized that chronic administration of agmatine along with morphine interferes with some step in these intracellular signal transduction pathways, thereby reducing dependence/withdrawal. In fact, one previous study reported that agmatine inhibited the increase in cAMP in morphine-exposed NG108-15 cells when the cells were challenged with naloxone.[65] Our recent report confirmed this finding in brain cortical slices of rats chronically exposed to morphine and agmatine.[37] We have also observed that agmatine inhibits the higher expression of TH during chronic morphine exposure in rat LC and striatum (S. Regunathan and F. Aricoglu, unpublished data, December 2004). These initial findings support our hypothesis, but further studies are required to investigate how agmatine regulate the downstream events of cAMP signaling.

Morphine Exposure and Endogenous Agmatine

While exogenous agmatine is clearly effective in modulating opiate analgesia/dependence, whether endogenous agmatine has a similar function is not known. It is important to note that all the brain structures that are involved in drugs of abuse—the VTA, the nucleus accumbens, the amygdala, and the LC—contain substantial agmatine immunoreactive neurons.[7] Agmatine and ADC activity are known to be regulated in certain other conditions, such as inflammatory neuropathic pain, ischemic stroke, and depression.[18,20,72,73] Our initial studies have indicated that agmatine levels and ADC activity are lower in the rat brain and other tissues after 3 days of exposure to morphine.[74] Studies on the in vivo release of agmatine during morphine exposure and withdrawal should provide some evidence for the role of endogenous agmatine in opiate drug abuse. However, the ultimate proof will be to show that increasing endogenous agmatine levels reduces symptoms of morphine withdrawal and decreasing these levels exacerbates symptoms of morphine withdrawal. Blocking the biosynthetic enzyme, ADC, could be the direct approach to reducing endogenous agmatine levels. Although no selective inhibitor of mammalian ADC is presently available, other means of decreasing ADC activity are feasible. For example, the cDNA sequence of mammalian ADC can be used to design RNA interference (small double-stranded RNA) to degrade ADC messenger RNA (mRNA), thereby lowering the expression of ADC activity and levels of agmatine. We have recently produced small interfering ribonucleic aid (siRNA) capable of reducing ADC mRNA levels and agmatine production in cultured neurons and glial cells.[75]

Another approach that is currently being used successfully is blocking the action of endogenous agmatine by selective agmatine antibodies. In a recent study, Fairbanks et al showed that antiagmatine immunoglobulin G (IgG), but not normal IgG, reversed exogenous agmatine-mediated, but not MK801-mediated, inhibition of NMDA-evoked behavior in mice and induced tolerance to opioid agonists at lower doses.[76] These findings were interpreted to mean that sequestration of endogenous agmatine by agmatine-selective IgG increases the susceptibility to tolerance induced by opioid agonists. This strategy has been used previously[77] to show that an antiserum raised against [Leu5]enkephalin prevents the increase in morphine potency induced by exogenous i.c.v. administration of [Leu5]enkephalin. These results validate the approach of determining the physiological effects of inactivating endogenous agmatine by administering exogenous antisera.

Obviously, further studies using the approach of increasing or decreasing endogenous agmatine are required to establish the proof-of-concept that agmatine is an endogenous protective molecule against morphine/opiate dependence/withdrawal and that increasing its levels in the brain benefits the system. Such observations will also provide a basis for the use of drugs to increase endogenous agmatine levels as a way to potentiate morphine analgesia and reduce morphine dependence/tolerance. Although agmatine is effective in animal models, it is unlikely to be a useful drug candidate because of its rapid metabolism, high turnover, and poor penetration into the brain. Frequent administration of high doses of exogenous agmatine is required to observe the effect in animal models. Thus, increasing endogenous agmatine by other means will be a valuable way to sustain higher levels of agmatine in the brain. One approach would be the use of inhibitors of agmatinase, the major degradative enzyme for agmatine in the brain.[78] The inhibitors of mammalian agmatinase are currently being evaluated,[34] and these initial efforts have identified a certain class of compounds as potential targets. It is important to identify selective agmatinase inhibitors since many structural analogs of agmatine are also potent inhibitors of NMDA receptors. For example, arcaine (diguanido butane), a potent inhibitor of agmatinase,[34] is a well-known NMDA antagonist.[45] From our initial screening of selective compounds, we have identified 3-aminoprpopyl guanidine as a potent inhibitor of agmatinase in vitro with no binding to NMDA receptors.[34] Further studies are under way to determine whether agmatinase inhibitors can actually increase endogenous agmatine levels in the brain in vivo. The use of agmatinase inhibitors along with exogenous agmatine will be a very useful approach to sustaining higher levels of agmatine in the brain.

Conclusions

One of the most fascinating aspects of agmatine, an endogenous molecule, is its ability to potentiate the analgesic effect of morphine while also reducing morphine dependence and withdrawal symptoms. At the same time, agmatine has absolutely no effect in naive animals on behavior, locomotion, or cardiovascular functions. Here, therefore, we have the opportunity to manipulate a system that is activated only when the normal homeostasis of the brain/cells/neurons is altered, for example, in the hyperexcitable state after chronic morphine exposure. Moreover, as agmatine has multiple molecular targets with low affinity and, thus, is easily reversible in functional actions with no toxic effects, it has tremendous therapeutic potential. The use of agmatine by itself or along with selective agmatinase inhibitors will be a valuable therapeutic approach for several targets, including ischemic injury, convulsive seizures, and opiate analgesia with reduced risk of dependence.

References

1. Li G, Regunathan S, Barrow CJ, Eshraghi J, Cooper R, Reis DJ. Agmatine: an endogenous clonidine-displacing substance in the brain. *Science*. 1994;263:966-969.

2. Piletz JE, Chikkala DN, Ernsberger P. Comparison of the properties of agmatine and endogenous clonidine-displacing substance at imidazoline and alpha-2 adrenergic receptors. *J Pharmacol Exp Ther*. 1995;272:581-587.

3. Pinthong D, Hussain JF, Kendall DA, Wilson VG. Comparison of the interaction of agmatine and crude methanolic extracts of bovine lung and brain with alpha 2-adrenoceptor binding sites. *Br J Pharmacol*. 1995;115:689-695.

4. Pinthong D, Wright IK, Hanmer C, et al. Agmatine recognizes alpha 2-adrenoceptor binding sites but neither activates nor inhibits alpha 2-adrenoceptors. *Naunyn Schmiedebergs Arch Pharmacol*. 1995;351:10-16.

5. Reis DJ, Regunathan S. Is agmatine a novel neurotransmitter in brain? *Trends Pharmacol Sci*. 2000;21:187-193.

6. Wang H, Regunathan S, Youngson C, Bramwell S, Reis DJ. An antibody to agmatine localizes the amine in bovine adrenal chromaffin cells. *Neurosci Lett*. 1995;183:17-21.

7. Otake K, Ruggiero DA, Regunathan S, Wang H, Milner TA, Reis DJ. Regional localization of agmatine in the rat brain: an immunocytochemical study. *Brain Res*. 1998;787:1-14.

8. Yang X-C, Reis DJ. Agmatine selectively blocks the NMDA subclass of glutamate receptor channels in cultured mouse hippocampal neurons. *J Pharmacol Exp Ther*. 1999;288:544-549.

9. Gorbatyuk OS, Milner TA, Wang G, Regunathan S, Reis DJ. Localization of agmatine in vasopressin and oxytocin neurons of the rat hypothalamic paraventricular and supraoptic nuclei. *Exp Neurol*. 2001;171:235-245.

10. Li G, Regunathan S, Reis DJ. Agmatine is synthesized by a mitochondrial arginine decarboxylase in rat brain. *Ann N Y Acad Sci*. 1995;763:325-329.

11. Lortie MJ, Novotny WF, Peterson OW, et al. Agmatine, a bioactive metabolite of arginine. *J Clin Invest*. 1996;97:413-420.

12. Zhu MY, Iyo A, Piletz JE, Regunathan S. Expression of human arginine decarboxylase, the biosynthetic enzyme for agmatine. *Biochim Biophys Acta*. 2004;1670:156-164.

13. Sastre M, Regunathan S, Reis DJ. Agmatinase activity in rat brain: a metabolic pathway for the degradation of agmatine. *J Neurochem*. 1996;67:1761-1765.

14. Holt A, Baker GB. Metabolism of agmatine (clonidine-displacing substance) by diamine oxidase and the possible implications for studies of imidazoline receptors. *Prog Brain Res*. 1995;106:187-197.

15. Olmos G, DeGregorio-Rocasolano N, Regalado MP, et al. Protection by imidazol(ine) drugs and agmatine of glutamate-induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor. *Br J Pharmacol*. 1999;127:1317-1326.

16. Zhu MY, Piletz JE, Halaris A, Regunathan S. Effect of agmatine against cell death induced by NMDA and glutamate in neurons and PC12 cells. *Cell Mol Neurobiol*. 2003;23:865-872.

17. Gilad GM, Gilad VH. Accelerated functional recovery and neuroprotection by agmatine after spinal cord ischemia in rats. *Neurosci Lett*. 2000;296:97-100.

18. Gilad GM, Gilad VH, Rabey JM. Arginine and ornithine decarboxylation in rodent brain—coincidental changes during development and after ischemia. *Neurosci Lett*. 1996;216:33-36.

19. Kim JH, Yenari MA, Giffard RG, Cho SW, Park KA, Lee JE. Agmatine reduces infarct area in a mouse model of transient focal cerebral ischemia and protects cultured neurons from ischemia-like injury. *Exp Neurol*. 2004;189:122-130.

20. Fairbanks CA, Schreiber KL, Brewer KL, et al. Agmatine reverses pain induced by inflammation, neuropathy, and spinal cord injury. *Proc Natl Acad Sci USA*. 2000;97:10584-10589.

21. Feng Y, Piletz JE, Leblanc MH. Agmatine suppresses nitric oxide production and attenuates hypoxic-ischemic brain injury in neonatal rats. *Pediatr Res*. 2002;52:606-611.

22. Bence AK, Worthen DR, Stables JP, Crooks PA. An in vivo evaluation of the antiseizure activity and acute neurotoxicity of agmatine. *Pharmacol Biochem Behav*. 2003;74:771-775.

23. Su RB, Wei XL, Zheng JQ, Liu Y, Lu XQ, Li J. Anticonvulsive effect of agmatine in mice. *Pharmacol Biochem Behav*. 2004;77:345-349.

24. Aricioglu F, Kan B, Yillar O, Korcegez E, Berkman K. Effect of agmatine on electrically and chemically induced seizures in mice. *Ann N Y Acad Sci*. 2003;1009:141-146.

25. Kolesnikov Y, Jain S, Pasternak GW. Modulation of opioid analgesia by agmatine. *Eur J Pharmacol*. 1996;296:17-22.

26. Aricioglu-Kartal F, Uzbay IT. Inhibitory effect of agmatine on naloxane-precipitated abstinence syndrome. *Life Sci*. 1997;61:1775-1781.

27. Fairbanks CA, Brewer KL, Stone LS, et al. The behavioral and neuroprotective effects of agmatine in different models of pain and neuronal injury in rodents. *Soc Neurosci Abstr*. 1998;24:1253.

28. Li J, Li X, Pei G, Qin BY. Agmatine inhibited tolerance to and dependence on morphine in guinea pig ileum in vitro. *Zhongguo Yao Li Xue Bao*. 1998;19:564-568.

29. Nguyen HO, Goracke-Postle CJ, Kaminski LL, Overland AC, Morgan AD, Fairbanks CA. Neuropharmacokinetic and dynamic studies

of agmatine (decarboxylated arginine). *Ann N Y Acad Sci*. 2003;1009:82-105.

30. Arteni NS, Lavinsky D, Rodrigues AL, Frison VB, Netto CA. Agmatine facilitates memory of an inhibitory avoidance task in adult rats. *Neurobiol Learn Mem*. 2002;78:465-469.

31. Lavinsky D, Arteni NS, Netto CA. Agmatine induces anxiolysis in the elevated plus maze task in adult rats. *Behav Brain Res*. 2003;141:19-24.

32. Zomkowski AD, Hammes L, Calixto JB, Lin J, Santos AR, Rodrigues AL. Agmatine produces antidepressant-like effects in two models of depression in mice. *Neuroreport*. 2002;13:387-391.

33. Sauve Y, Reader TA. Effects of alpha-methyl-p-tyrosine on monoamines and catecholamine receptors in rat cerebral cortex and neostriatum. *Neurochem Res*. 1988;13:807-815.

34. Dias Elpo Zomkowski A, Oscar Rosa A, Lin J, Santos AR, Calixto JB, Lucia Severo Rodrigues A. Evidence for serotonin receptor subtypes involvement in agmatine antidepressant like-effect in the mouse forced swimming test. *Brain Res*. 2004;1023:253-263.

35. Li J, Li X, Pei G, Qin BY. Effects of agmatine on tolerance to and substance dependence on morphine in mice. *Zhongguo Yao Li Xue Bao*. 1999;20:232-238.

36. Uzbay IT, Yesilyurt O, Celik T, Ergun H, Isimer A. Effects of agmatine on ethanol withdrawal syndrome in rats. *Behav Brain Res*. 2000;107:153-159.

37. Aricioglu F, Means A, Regunathan S. Effect of agmatine on the development of morphine dependence in rats: potential role of cAMP system. *Eur J Pharmacol*. 2004;504:191-197.

38. Aricioglu F, Paul IA, Regunathan S. Agmatine reduces only peripheral-related behavioral signs, not the central signs, of morphine withdrawal in nNOS deficient transgenic mice. *Neurosci Lett*. 2004;354:153-157.

39. Morgan AD, Campbell UC, Fons RD, Carroll ME. Effects of agmatine on the escalation of intravenous cocaine and fentanyl self-administration in rats. *Pharmacol Biochem Behav*. 2002;72:873-880.

40. Aricioglu F, Korcegez E, Bozkurt A, Ozyalcin S. Effect of agmatine on acute and mononeuropathic pain. *Ann N Y Acad Sci*. 2003;1009:106-115.

41. Onal A, Delen Y, Ulker S, Soykan N. Agmatine attenuates neuropathic pain in rats: possible mediation of nitric oxide and noradrenergic activity in the brainstem and cerebellum. *Life Sci*. 2003;73:413-428.

42. Yu CG, Fairbanks CA, Wilcox GL, Yezierski RP. Effects of agmatine, interleukin-10, and cyclosporin on spontaneous pain behavior after excitotoxic spinal cord injury in rats. *J Pain*. 2003;4:129-140.

43. Santos AR, Gadotti VM, Oliveira GL, et al. Mechanisms involved in the antinociception caused by agmatine in mice. *Neuropharmacology*. 2005;48:1021-1034.

44. Bradley KJ, Headley PM. Effect of agmatine on spinal nociceptive reflexes: lack of interaction with alpha2-adrenoceptor or mu-opioid receptor mechanisms. *Eur J Pharmacol*. 1997;331:133-138.

45. Reynolds IJ. Arcaine uncovers dual interactions of polyamines with the n-methyl-d-aspartate receptor. *J Pharmacol Exp Ther*. 1990;255:1001-1007.

46. Gibson DA, Harris BR, Rogers DT, Littleton JM. Radioligand binding studies reveal agmatine is a more selective antagonist for a polyamine-site on the NMDA receptor than arcaine or ifenprodil. *Brain Res*. 2002;952:71-77.

47. Askalany AR, Yamakura T, Petrenko AB, Kohno T, Sakimura K, Baba H. Effect of agmatine on heteromeric N-methyl-d-aspartate receptor channels. *Neurosci Res*. 2005;52:387-392.

48. Trujillo KA, Akil H. Inhibition of opiate tolerance by non-competitive N-methyl-D-aspartate receptor antagonists. *Brain Res*. 1994;633:178-188.

49. Jurkiewicz NH, do Carmo LG, Hirata H, da Costa Santos W, Jurkiewicz A. Functional properties of agmatine in rat vas deferens. *Eur J Pharmacol*. 1996;307:299-304.

50. Gatti PJ, Hill KJ, Da Silva AM, Norman WP, Gillis RA. Central nervous system site of action for the hypotensive effect of clonidine in the cat. *J Pharmacol Exp Ther*. 1988;245:373-380.

51. Hansson BG, Hokfelt B. Changes in blood pressure, plasma catecholamines and plasma renin activity during and after treatment with tiamenidine and clonidine. *Br J Clin Pharmacol*. 1981;11:73-77.

52. Hieble JP, Kolpak DC. Mediation of the hypotensive action of systemic clonidine in the rat by alpha 2-adrenoceptors. *Br J Pharmacol*. 1993;110:1635-1639.

53. Sun MK, Regunathan S, Reis DJ. Cardiovascular responses to agmatine, a clonidine-displacing substance, in anesthetized rat. *Clin Exp Hypertens*. 1995;17:115-128.

54. Szabo B, Urban R, Limberger N, Starke K. Cardiovascular effects of agmatine, a "clonidine-displacing substance", in conscious rabbits. *Naunyn Schmiedebergs Arch Pharmacol*. 1995;351:268-273.

55. Raasch W, Schafer U, Qadri F, Dominiak P. Agmatine, an endogenous ligand at imidazoline binding sites, does not antagonize the clonidine-mediated blood pressure reaction. *Br J Pharmacol*. 2002;135:663-672.

56. Tokuyama S, Zhu H, Oh S, Ho IK, Yamamoto T. Further evidence for a role of NMDA receptors in the locus coeruleus in the expression of withdrawal syndrome from opioids. *Neurochem Int*. 2001;39:103-109.

57. Wang G, Gorbatyuk O, Dayanithi G, et al. Evidence for endogenous agmatine in hypothalamo-neurohypophysial tract and its modulation on vasopressin release and Ca2+ channels. *Brain Res*. 2002;932:25-36.

58. Zheng JQ, Weng XC, Gai XD, Li J, Xiao WB. Mechanism underlying blockade of voltage-gated calcium channels by agmatine in cultured rat hippocampal neurons. *Acta Pharmacol Sin*. 2004;25:281-285.

59. Feng Y, Leblanc MH, Regunathan S. Agmatine reduces extracellular glutamate during pentylenetetrazole-induced seizures in rat brain: a potential mechanism for the anticonvulsive effects. *Neurosci Lett*. 2005;390:129-133.

60. Regunathan S, Youngson C, Raasch W, Wang H, Reis DJ. Imidazoline receptors and agmatine in blood vessels: a novel system inhibiting vascular smooth muscle proliferation. *J Pharmacol Exp Ther*. 1996;276:1272-1282.

61. Satriano J, Matsufuji S, Murakami Y, et al. Agmatine suppresses proliferation by frameshift induction of antienzyme and attenuation of cellular polyamine levels. *J Biol Chem*. 1998;273:15313-15316.

62. Abe K, Abe Y, Saito H. Agmatine suppresses nitric oxide production in microglia. *Brain Res*. 2000;872:141-148.

63. Satriano J, Schwartz D, Ishizuka S, et al. Suppression of inducible nitric oxide generation by agmatine aldehyde: beneficial effects in sepsis. *J Cell Physiol*. 2001;188:313-320.

64. Regunathan S, Piletz JE. Regulation of inducible nitric oxide synthase and agmatine synthesis in macrophages and astrocytes. *Ann N Y Acad Sci*. 2003;1009:20-29.

65. Li J, Li X, Pei G, Qin BY. Influence of agmatine in adaptation of cAMP signal transduction system of opiate receptors. *Zhongguo Yao Li Xue Bao*. 1999;20:592-596.

66. Nestler EJ. Molecular neurobiology of addiction. *Am J Addict*. 2001;10:201-217.

67. Copeland RL, Pradhan SN, Dillon-Carter O, Chuang DM. Rebound increase of basal cAMP level in NG108-15 cells during chronic morphine treatment: effects of naloxane and chloramphenicol. *Life Sci*. 1989;44:1107-1116.

68. Mehta CS, Strada SJ. Effects of acute and continuous administration of morphine on the cAMP response induced by norepinephrine in rat brain slices. *Life Sci*. 1994;55:35-42.

69. Avidor-Reiss T, Bayewitch M, Levy R, Matus-Leibovitch N, Nevo I, Vogel Z. Adenylylcyclase supersensitization in mu-opioid receptor-transfected Chinese hamster ovary cells following chronic opioid treatment. *J Biol Chem*. 1995;270:29732-29738.

70. Guitart X, Nestler EJ. Second messenger and protein phosphorylation mechanisms underlying opiate addiction: studies in the rat locus coeruleus. *Neurochem Res*. 1993;18:5-13.

71. Lane-Ladd SB, Pineda J, Boundy VA, et al. CREB (cAMP response element-binding protein) in the locus coeruleus: biochemical, physiological, and behavioral evidence for a role in opiate dependence. *J Neurosci*. 1997;17:7890-7901.

72. Feng Y, Leblanc MH. Effect of agmatine on the time course of brain inflammatory cytokines after injury in rat pups. *Ann N Y Acad Sci*. 2003;1009:152-156.

73. Halaris A, Zhu H, Feng Y, Piletz JE. Plasma agmatine and platelet imidazoline receptors in depression. *Ann N Y Acad Sci*. 1999;881:445-451.

74. Aricioglu-Kartal F, Regunathan S. Effect of chronic morphine treatment on the biosynthesis of agmatine in rat brain and other tissues. *Life Sci*. 2002;71:1695-1701.

75. Iyo AH, Zhu MY, Ordway GA, Regunathan S. Expression of arginine decarboxylase in brain regions and neuronal cells. *J Neurochem*. 2006;96:1042-1050.

76. Fairbanks CA, Kaminski LL, Nguyen HO, et al. Pretreatment with antisera raised against agmatine sensitizes mice to plasticity-mediated events [abstract]. *Soc Neurosci Abstr [serial online]*. 2001;27:.

77. Vanderah TW, Wild KD, Takemori AE, et al. Modulation of morphine antinociception by swim-stress in the mouse: involvement of supraspinal opioid delta-2 receptors. *J Pharmacol Exp Ther*. 1993;267:449-455.

78. Huang M-J, Regunathan S, Botta M, et al. Structure-activity analysis of guanidine group in agmatine for brain agmatinase. *Ann N Y Acad Sci*. 2003;1009:52-63.